Temperature gradient stimulation for cell division in C. Elegans Embryos on chip

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TEMPERATURE GRADIENT STIMULATION FOR CELL DIVISION IN C. ELEGANS EMBRYOS ON CHIP

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ABSTRACT

This paper reports on a new microfluidic device for temperature stimulation of cell in *in-vitro* culture. Micro-electrodes in a meander shape are embedded into the microfluidic channels to generate either a temperature gradient through the culture chamber or a local heat spot under specific cells. One promising application is the control of cell division rate. Here we present first results of the synchronization of cell division in a two-cell stage embryos of C. Elegans.

KEYWORDS: microfluidic, temperature gradient, micro-electrodes, cell division

INTRODUCTION

In developmental biology, in-vitro methods are essential to observe, identify and understand important processes such as cell division, gene transcription or cell-to-cell communication. In the last decades, microtechnology has offered the opportunity to develop new experimental platforms enabling the precise control of the cell environment [1]. With our device, we intend to control the cell division rate by a change in temperature. Compared with published method based on laminar-flow [2], this new platform presents several advantages such as the temperature-gradient is created within few seconds by simply switching on the current source, thus avoiding the switching of fluids and consequently, the creation of air bubbles and shear stress. In addition, it is possible to carry out multiple-assays in parallel.

One application of this new tool is the synchronization of cell division in C. Elegans embryos. In fact, in the two-cell stage embryos, the larger anterior cell divides before the smaller posterior one [3]. This asynchronous process is due to asymmetry in protein pattern across the embryo, with PAR-3-Par-6-PKC-3 complex on the anterior part and PAR-2 on the posterior part which tend to position the spindle asymmetrically [4]. To understand the role of this asynchronous process in the development, the slower cell will be stimulated by heat to divide at the same time as the faster cell.

EXPERIMENTAL

The device is composed of a microfluidic channel network sealed by a glass cover slip where electrodes are patterned (Figure 1b). The channels are molded in poly(dimethylsiloxane) (PDMS) from silicon wafers structured by standard microfabrication processes. Titanium-platinum micro-electrodes have been deposited by evaporation on the glass slide. Platinum has a resistivity linearly dependent on the temperature and thus, can be used as a temperature sensor. Choosing a four point electrode design (Figure 2c) allows us to inject the current in the platinum meanders to produce heat by Joule effect and to measure simultaneously the tension across the heating part of the electrode in order to determine the resistance of platinum and thus, the temperature in this area.

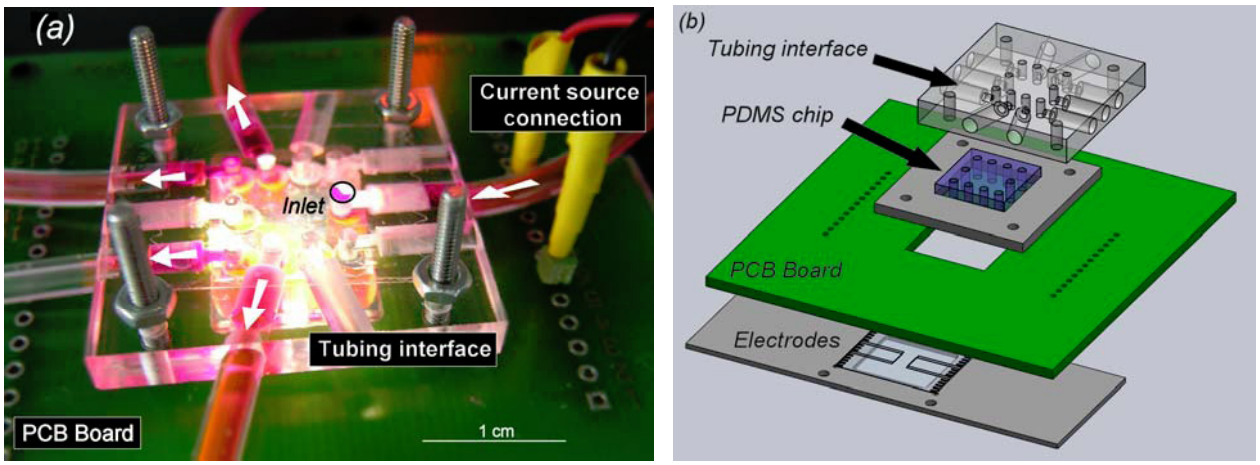

Figure 1: (a) Photograph of the complete set-up: tubing interface to connect micro-channels to syringe pump, current source connection on the PCB board to heat electrodes (b) 3D view of all the components of the set-up.

For this project, specific features of the microfluidic channels have been created to trap embryos near the electrode. The channels are 40 µm height and 40 µm width, fitting the embryo size, and constricted by pillars of 20 µm diameter (Figure 2). Moreover, four channels are implemented on one chip to perform experiments in parallel.

From a user point of view, the experiment has to be set up within few minutes, before the embryos turn to a four-cell stage. The microfluidic network is preliminary filled with standard medium and special attention is paid to eliminate all air bubbles. Thanks to the single inlet (Figure 1a and Figure 2c), four to six embryos, collected in one worm, are in-

jected in one shot. By pressure-driven flow, the embryos are then driven and trapped at the specific position. Figure 2a shows a two-cell stage embryo with the right orientation; the smaller posterior cell facing the electrode where the gradient is created. Finally, the current source is switched on and the electrode starts to heat.

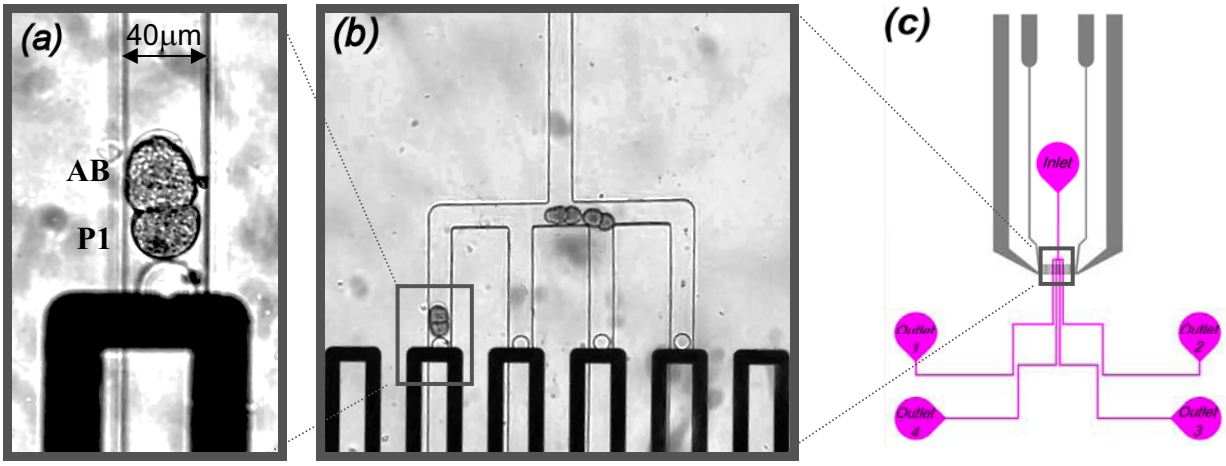

Figure 2: (a) A two-cell stage embryo trapped in the 40 µm-width channel near the electrode in the appropriate orientation. (b) Three C.Elegans embryos in the microfluidic network (c) Drawing of the microchannels (pink) with one inlet and four outlets and the network of electrodes (grey)

RESULTS AND DISCUSSION

In order to estimate the gradient of temperature required for the synchronization, off-chip experiments have been carried out, measuring the cell cycle duration for the larger anterior cell (AB) and the smaller posterior cell (P1) in embryos kept at different temperatures (Figure 3). A first observation is that, outside of the range of 14°C to 28°C, the embryos are not viable. Concerning the cell cycle duration, AB and P1 cells present a same dependency on temperature, even if P1 has a general development slower than AB, about 2 minutes of delay, and is more sensitive at high temperature.

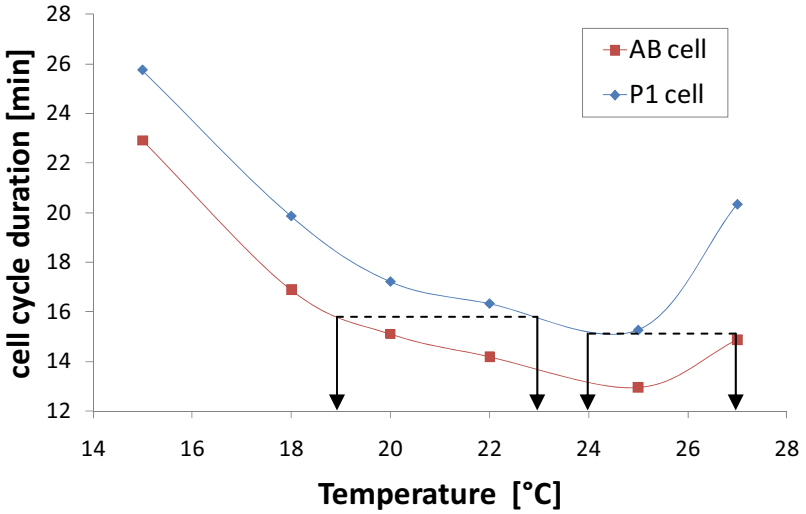

Figure 3: Cell cycle duration in a two-cell stage embryo of C. Elegans in function of the temperature (measurement have been carried out on two to five embryos)

Based on these results, two strategies for synchronization are possible. First, AB cell is kept at room temperature below 20°C, such as many microscope rooms. P1 cell has to be heated up to a temperature higher of 4°C to 5°C than the temperature of AB cell. Alternatively, if P1 cell is kept at a room temperature of 24°C, AB cell has to reach a temperature of 27°C to divide at the same time as P1. This last method is more hazardous than the first one because the embryo reaches the limit temperature for viability. In both cases, the challenge will be to establish a temperature gradient up to 5°C across a 50µm-long embryo to synchronize AB and P1 cell division.

Before introducing embryos in the device, a characterization of the heaters has been carried out to determine the optimal conditions to generate the correct temperature gradient. As a first attempt, Rhodamine B, a temperature dependent fluorescent dye [5] has been chosen to evaluate the temperature gradient. The microfluidic channels are filled with a solution of 0.1 mmol/L of Rhodamine B and the current source is switched on.

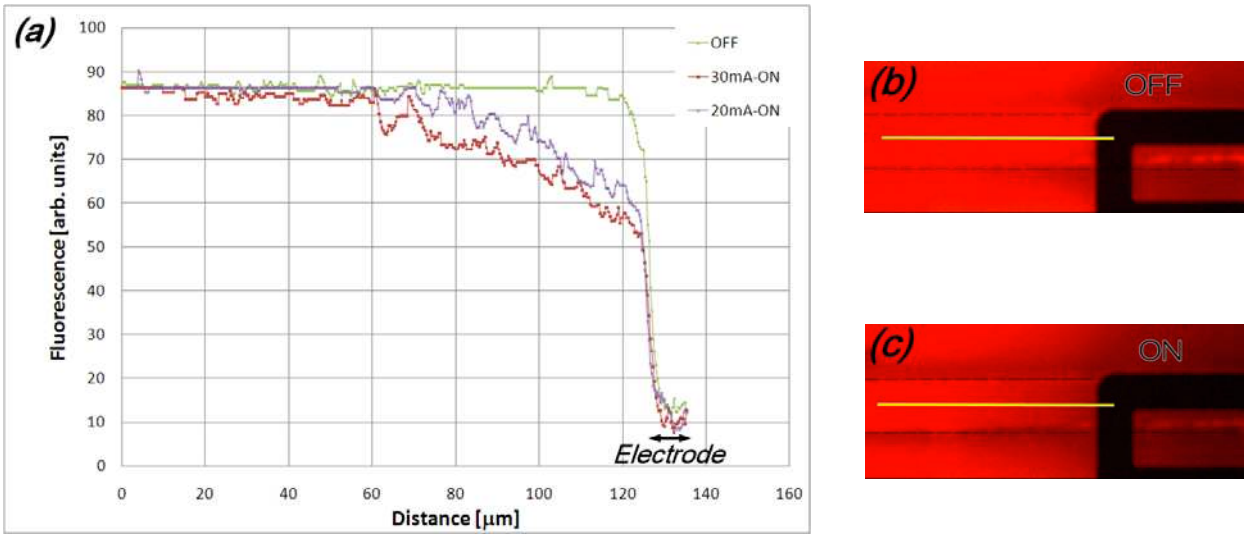

Figure 4: (a) The fluorescence intensity of Rhodamine B along the channel (yellow line in photographs (b) and (c)) is plotted. A clear decrease in fluorescence intensity is observed in the 50 µm away from the electrodes when a current of 20 mA and 30 mA (c) is applied.

In Figure 4, the results show that a significant decrease of fluorescence appears when the current is injected in the electrodes, meaning that heat occurs. The temperature gradient is established within 50 µm away from the electrode, covering nearly the length of the embryo (50 µm) trapped by the pillars.

In future experiments, Rhodamine B intensity will be calibrated in function of temperature and PDMS channels will be coated to prevent dye adsorption in order to measure precisely the temperature gradient generated.

CONCLUSION

These preliminary results prove that our microfluidic device can generate a temperature gradient over a single embryo thanks to an efficient mechanical trapping. Numerical simulations are currently performed in parallel of the Rhodamine B calibration to find the optimal conditions to generate the temperature gradient required for synchronization.

In conclusion, we can say that this device opens a new experimental way in developmental biology allowing a better control on cell division rate with no genetic modification.

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